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ORIGINAL ARTICLE

Phytochemical Profiling GC-MS / LCMS Analysis of Punarnava Eye Drops in the Management of Allergic Conjunctivitis

Nishu Yadav, Shalaka More, Manjiri Keskar and Sobha Yadav
Department of Shalakya Tantra, Parul Institute of Ayurved, Parul University, Vadodara, Gujarat.
***Corresponding Author's**: Email: vdshalakapawar@gmail.com

ABSTRACT
Punarnava (Boerhavia diffusa) and honey are two extensively utilized medicinal agents in Ayurveda, the traditional Indian system of medicine, known for their anti-inflammatory, rejuvenating, and soothing properties. The present study aimed to evaluate the phytochemical constituents of Punarnava a polyherbal churna formulated in equal proportions using with honey, specifically for its potential application in the management of Vataja Abhishyanda—a condition of the eye characterized by watering discharge, Itching, foreign body sensation, congestion and pain due to vitiated Vata dosha. Preliminary phytochemical screening of the prepared formulation revealed the presence of several bioactive compounds, including alkaloids, saponins, polyphenols, tannins, steroids, carbohydrates, and proteins, which are known to contribute to its therapeutic efficacy. Furthermore, the formulation was standardized using High-Performance Thin Layer Chromatography (HPTLC) fingerprinting, establishing a characteristic chemical profile. This HPTLC fingerprint may serve as a reliable tool for the authentication, quality control, and reproducibility of the polyherbal formulation intended for use in Vataja Abhishyanda treatment
Keywords: *Punarnava, honey, polyherbal formulation, phytochemical analysis, Bioactive compounds, HPTLC, Vataja Abhisyanda*

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INTRODUCTION

Punarnava (*Boerhavia diffusa*) eye drops are a traditional Ayurvedic preparation, recognized for their therapeutic potential in the management of Vataja Abhishyanda [1. 2]—an ocular condition marked by watering discharge, Itching, foreign body sensation, congestion and pain resulting from the aggravation of Vata dosha. Based on classical Ayurvedic principles and prior clinical observations suggesting beneficial effects of Punarnava in eye disorders. This formulation, developed as a medicated herbal eye drop using standardized extracts of *Boerhavia diffusa*, represents a refined and targeted intervention for Vataja-related ocular conditions. To validate and standardize the formulation, the study also included pharmacognostical analysis, preliminary phytochemical screening, and assessment of physico-chemical parameters. Additionally, High-Performance Thin Layer Chromatography (HPTLC) was employed to generate a characteristic fingerprint profile for Punarnava eye drops. This fingerprint can serve as a reliable reference for ensuring the quality, authenticity, and reproducibility of the formulation intended for clinical use in Vataja Abhishyanda [3].

MATERIAL AND METHODS

Collection of Raw Materials

The herbs used in punarnava eye drop include:

- **Punarnava (***Boerhavia diffusa*) [4-6]
- **Honey**

The R&D Laboratory at Shreedhareeyam Farm herbs India Pvt. Ltd. has undertaken the formulation of a sterile herbal eye drop incorporating the ingredients of Punarnava Rasakriya [7], a classical

Ayurvedic preparation. This formulation includes key components such as Punarnava (*Boerhavia diffusa*) and honey.

The Punarnava (*Boerhavia diffusa*) used in the eye drop's development was authenticated by a botanist.

Table No. 1 : Contents used in Punarnava Eye Drops

No	Botanical name	Sanskrit name	Part used	Quantity for 2.0 ltr
1.	*Boerhavia diffusa*	Punarnava	Roots	200.0 g
2.	Honey	Madhu	-	74 g

Pharmacodynamics of Punarnava Eye Drops

The pharmacological action and physiological effects of a drug are primarily influenced by its fundamental physicochemical attributes, namely Rasa (taste), Guna (qualities), Veerya (potency), Vipaka (post-digestive effect), Karma (action), and Prabhava (specific effect). These characteristics interact with the body's Doshas, guiding the drug's Doshakarma (action on Doshas), which helps to correct imbalances and restore Doshic equilibrium. The pharmacodynamics of the selected formulation, Punarnava eye drops, can be understood in this context.

Table No. 2 Properties of Punarnava and Honey

Dravya	***Rasa***	***Guna***	***Virya***	***Vipaka***	***Doshshamkta***
Punarnava	Madhura, tikta, kashaya	Laghu, ruksha	ushna	madhura	Vata-Kapha
Honey	Madhura, kashaya	Guru, ruksha	sheeta	-	Kapha-pitta

Method of Preparation of Punarnava Eye Drops

Punarnava (*Boerhavia diffusa*) was cleaned, washed, dried, and ground into a fine powder. A total of 200 grams of this powder was soaked in 3 liters of distilled water overnight. The mixture was then refluxed for 3 hours, allowed to cool, and filtered. The resulting filtrate was distilled, yielding approximately 1.5 liters of distillate.

To this distillate, 74.0 grams of honey [8] (7.4%) was added with continuous stirring. The pH of the solution was adjusted to approximately 6.921 using 0.2 N sodium hydroxide solution. Borax was used to buffer the solution and maintain a near-neutral pH. Benzalkonium chloride solution of pharmaceutical grade was added as a preservative, achieving a final concentration of 0.01% in the eye drop formulation. To ensure sterility, the solution was filtered through filter papers ranging from 2 µm to 0.2 µm. The sterile solution was then filled into sterile 5 ml dropper bottles and subjected to sterility testing in accordance with standard Protocols Test Report [9].

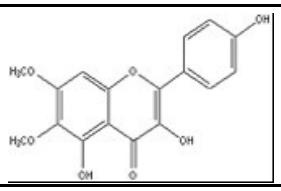
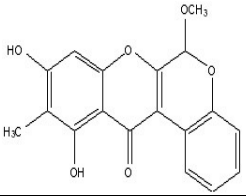
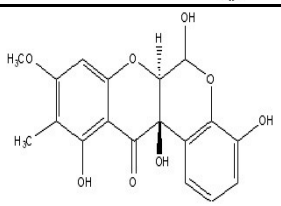
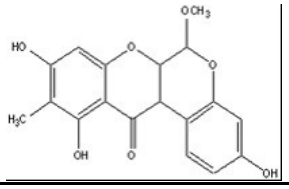


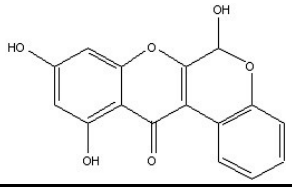
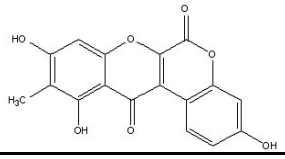
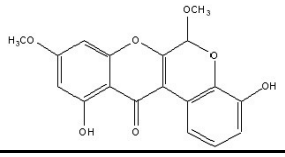
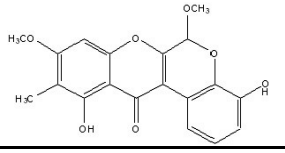
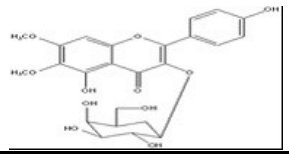
Photo No. 1 The eye drops preparations were prepared at Government of India, GMP certified pharmacy Shreedhareeyam, Kerala with all asceptic precautions.

Table No. 3 Chemical components of *Boerhavia diffusa* [10]

Class	Compounds
Alkaloid	Punarnavine
Rotenoids	Boeravine A-F Hypoxanthine 9-L-arabinofuranoside Hentriacontane, β-sitosterol and ursolic acid
Glycoside	Punarnavoside C-methylflavone5,7-dihydroxy-3',4'dimethoxy-6,8-dimethylflavone 40β-ecdysone, triacontane β-sitosterol-β-D-glucoside
Acids	tetracosanoic, hexacosanoic, stearic, palmitic, arachidic acids Boerhavin and boerhavic acid
Lignans	Liriodendrin syringaresinol mono-β-D-glucoside Glycoprotein
Lipids	5-methyleicos-4-ene Eicos-4-ene 4-methyloctadec-3-ene 4-methylnonadecylbenzene
Phenolic compounds	3,4-dihydroxy-5methoxycinnamoylrhamnoside Quercetin 3-O-rhamnosyl (1→6) galactoside (quercetin 3-O-robinobioside) Quercetin 3-O-(2''-rhamnosyl)robinobioside Kaempferol 3-O-(2''-rhamnosyl)robinobioside 3,5,4'-rihydroxy-6,7-dimethoxyflavone 3O-galactosyl(1→2)glucoside [eupalitin 3O-galactosyl(1→2)glucoside]Caffeoyltartaric acid Kaempferol 3-O-robinobioside eupalitin 3-O-galactoside Quercetin Kaempferol 6, 9, 11-Trihydroxy-6a12adehydrorotenoid (coccineone B)

Table No. 4 Important Phyto-Chemical Constituents and Mode of Action of *Punarnava*

Name	Chemical Constituents	Mode of action
Eupalitin [11]		Antioxidant
Boeravinone B [12]		Antistress agent,
Boeravinone C [13]		Immunostimulant
Boeravinone D [14]		Immunostimulant

Coccineone B [15]		Spasmolytic effects
Boeravinone F [16]		Immunostimulant
Boeravinone G [17]		Anti-oxidant effects
Boeravinone H [18]		Immunostimulant
Rotenoids [19]		Antioxidant, Diuretic, Antifibrinolytic

DRUGS ANALYSIS

Estimation of Quercetin in Punarnava Eye Drops Sample Chromatographic parameters

Table NO. 5 Showing the Chromatographic Parameter and Specifications

Parameter	**Specifications**
Instrument model	Shimadzu LC20AD equipped with Auto sampler
Detector	UV Detector
Wavelength	369nm
Column used	Shimadzu shim pack C18 Column (250mm*4.5µm*5µ)
Mobile Phase	Methanol (isocratic)
Flow Rate	1ml/min
Oven temperature	35°C
Injection volume	10µl
Retention time (Rt)	3.25 min

Preparation of Standard

A solution was prepared by dissolving 10 mg of quercetin in methanol and diluting it to a final volume of 10 mL using a volumetric flask, yielding a stock concentration of 1000 µg/mL. From this stock, further dilutions were carried out to obtain final concentrations of 2, 4, 6, 8, and 10 µg/mL.

Preparation of sample solution

From the prepared eye drop formulation, 1 mL of the sample was taken and mixed with 5 mL of methanol in a 10 mL volumetric flask. The mixture was then filtered using a 0.20 µm syringe filter and further diluted with methanol to the mark. The resulting solution was used for HPLC analysis.

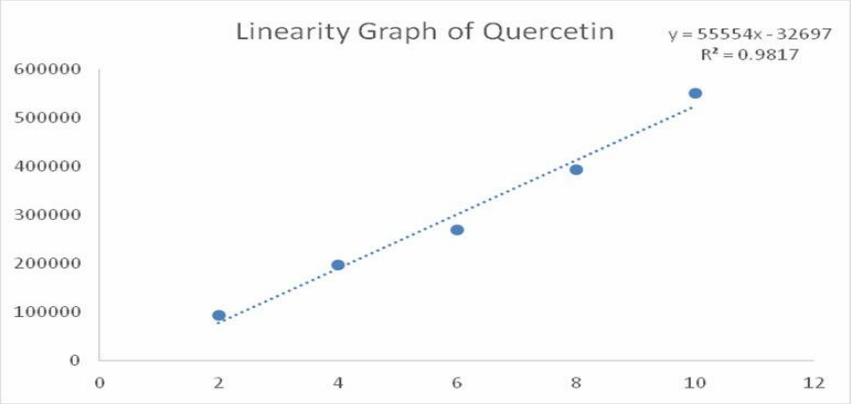
RESULTS AND DISCUSSION

HPTLC Analysis

Table No. 6 Linearity – Concentration and Peak Area of HPLC Studies of Punarnavadi Eye Drops

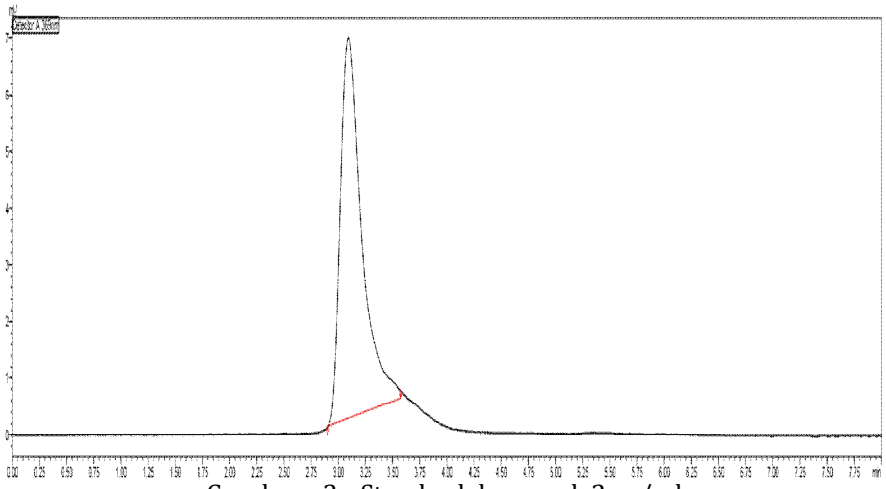
Concentration (µg/ml)	Peak Area
2	92990
4	196908
6	269255
8	393985
10	549989

Quercetin Linearity Graph



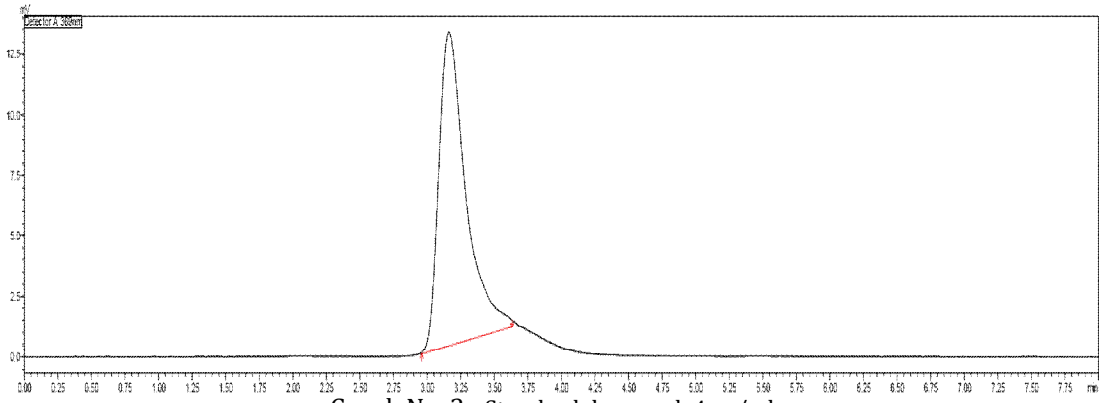
Graph no. 1 Standard Chromatograms:

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Sample Name:Q1 2
Sample ID:Q1 2



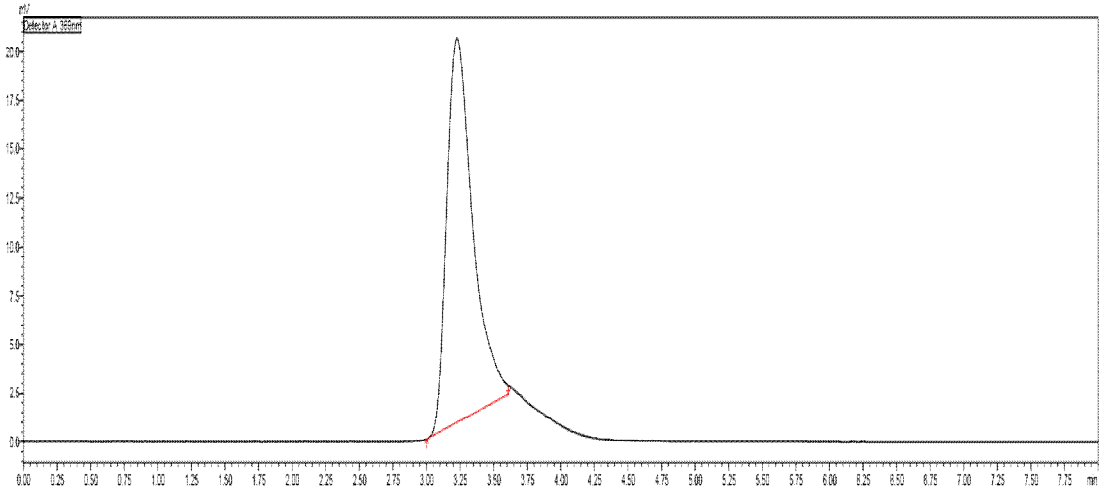
Graph no. 2 Standard drug peak 2 µg/ml

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Sample Name:Q1 4
Sample ID:Q1 4



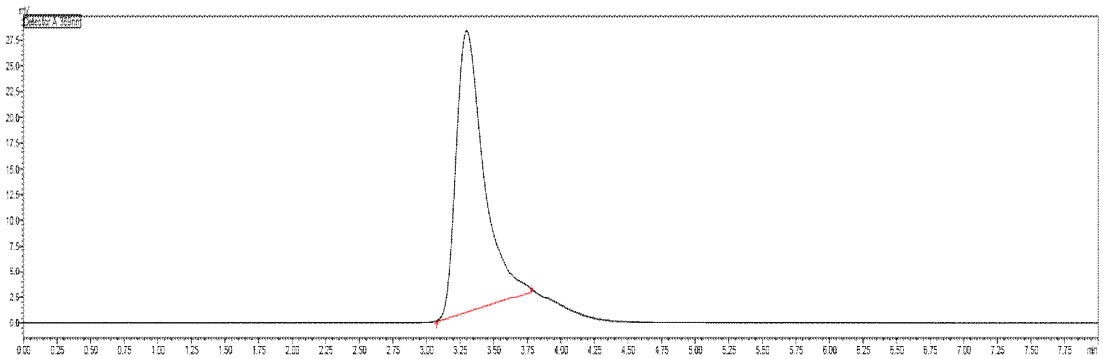
Graph No. 3 Standard drug peak 4 µg/ml

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Sample ID:Q1 6



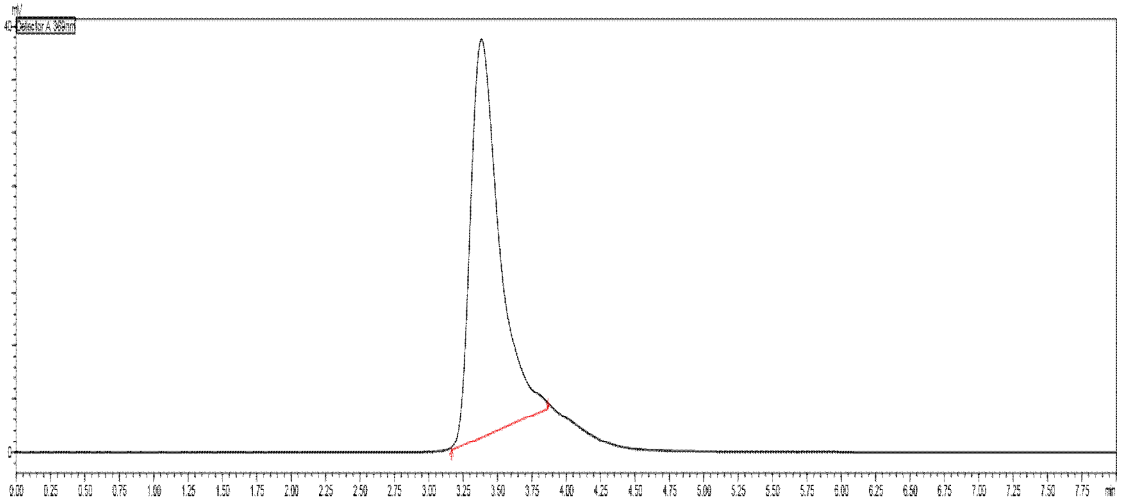
Graph No. 4 Standard drug peak 6 µg/ml

Datafile Name:Q1 8.lcd
Sample Name:Q1 8
Sample ID:Q1 8



Graph No. 5 Standard drug peak 8 µg/ml

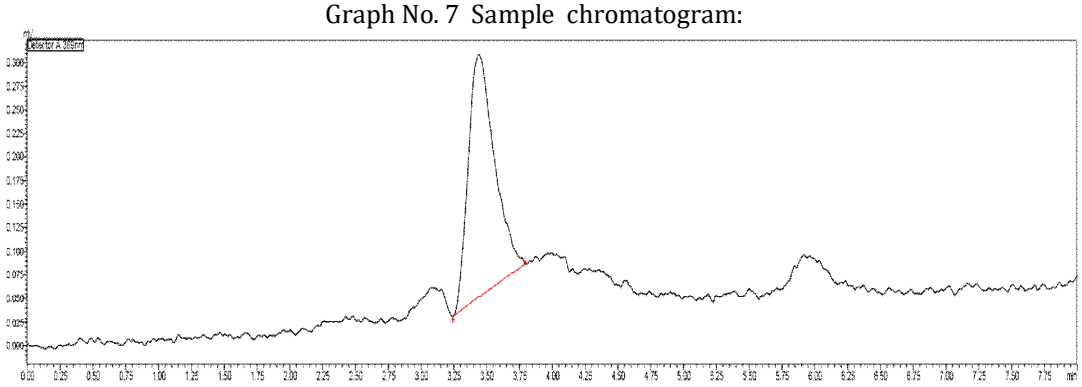
Datafile Name: Q1 10.lcd
SampleName: Q1 10
Sample ID: Q1 10



Graph.6 Standard drug peak 10 µg/ml

Datafile Name:Q1 S.lcd
Sample Name:Q1 S
Sample ID:Q1 S

Graph No. 7 Sample chromatogram:



REPORT

The estimated concentration of the Quercetin in given eye drop formulation was found to be 6.5µg/ml. Determination of Phytocomponents in Punarnava Eye drops by using LCMS/MS and GC-MS Spectrometry and Validation of Quercetin by UFLC Techniques.

Phyto-chemical Analysis

The phytoconstituents were identified using LC-MS/MS and GC-MS. Rotenoid, Kaempherol-3-o-rutinoside, Eupalitin-3-o-galactopyranoside, Isovitexin, Tetracosanoic, Boeravinone M, Boeravinone B, and Quinic acid were identified using LC-MS/MS. Three major constituents were identified using GC-MS: N-Hexadecanoic acid, 9, 12-Octadecadieonic acid, and Pentadecanoic acid. This can be used to determine the amount of Quercetin in a formulation and to qualify it.

Using LCMS/MS and GC-MS methods, the phytoconstituents present in *Boerhavia diffusa* were identified and identically matched the authenticated data. The procedure demonstrated linearity for various Quercetin concentrations. According to the ICH guidelines, the process was found to be precise, sensitive, and robust, with high reproducibility.

The Punarnava eye drop formulation was evaluated through phytochemical, chromatographic, organoleptic, and physicochemical analyses. The findings collectively confirm the presence of multiple bioactive constituents with potential relevance in allergic conjunctivitis (Vataja Abhishyanda).

Phytochemical Findings

Preliminary phytochemical screening demonstrated the presence of flavonoids, alkaloids, phenolics, saponins, steroids, carbohydrates, and proteins. These classes of compounds are widely reported in ***Boerhavia diffusa*** and are known for anti-inflammatory, antioxidant, and immunomodulatory effects. The findings align with earlier studies highlighting Punarnava's richness in rotenoids, flavonoids, and eupalitin derivatives known for antiallergic and tissue-protective properties.

Identification of Constituents (LC-MS/MS and GC-MS)

LC-MS/MS analysis identified major flavonoids and rotenoids including eupalitin-3-O-galactopyranoside, kaempferol-3-O-rutinoside, Boeravinone B, Boeravinone M, isovitexin, and quinic acid. These compounds are widely documented for antioxidant and anti-inflammatory mechanisms that support their role in reducing ocular irritation and allergic response.

GC-MS analysis further confirmed the presence of fatty acids such as N-hexadecanoic acid, 9,12-octadecadienoic acid, and pentadecanoic acid, which are known for soothing and membrane-stabilizing actions. These observations corroborate earlier phytochemical profiles of *Boerhavia diffusa* reported in classical and contemporary literature.

HPLC Quantification of Quercetin

HPLC analysis produced a clear linearity curve over the tested concentration range (2–10 µg/mL), indicating good method sensitivity and reliability for quercetin estimation. The sample peak corresponded to a quercetin content of 6.5 µg/mL**.** Quercetin is a well-established anti-inflammatory and antihistaminic flavonoid, and its quantified presence strengthens the therapeutic relevance of the formulation. Comparable concentrations have been reported in previous studies evaluating Punarnava extracts for ocular and systemic use .

Physicochemical and Organoleptic Parameters

The formulation exhibited clear clarity, pleasant odour, and a slight yellow colour, indicating good stability and aesthetic suitability for ophthalmic use. The pH (6.95) and viscosity values were within pharmaceutically acceptable ranges for ocular formulations, supporting compatibility with the ocular surface. Sterility tests were satisfactory, confirming adherence to ophthalmic preparation standards.

Table No. 7 Organoleptic study report of Punarnava Eye Drops

Sl. No.	Parameters Tested	Result	Method Used
1	Description	Slight yellow coloured clear Liquid	In-house Ref.
2	Size of Dropper	5mL	In-house Ref.
3	Colour of Cap	White	In-house Ref.
4	Clarity	Clear	In-house Ref.
5	Odour	Pleasant	In-house Ref.
6	pH	6.952	API, P. I, Vol.9, (first edition) page 114-115:2016
7	Specific Gravity at 25° C	1.025	API, P. II, Vol.4 (first edition) page 145:2017
8	Viscosity at 25° C	1.030	API, P. II, Vol.1, page 198:2007
9	Sterility	Passed	IP, Vol. I, page no. -66:2018

Overall Interpretation

The combined analytical findings confirm that the Punarnava eye drop formulation retains the characteristic phytochemical profile of *Boerhavia diffusa* along with honey, both of which are traditionally indicated for soothing inflamed or irritated eye tissues. The presence of flavonoids, rotenoids, phenolic acids, and fatty acids—supported by quantitative detection of quercetin—indicates strong antioxidant, anti-allergic, and protective properties. These results are consistent with past research showing Punarnava's efficacy in inflammatory and allergic ocular conditions.

The physicochemical results further validate that the formulation is safe, stable, and suitable for ophthalmic administration, supporting its potential use in managing Vataja Abhishyanda (allergic conjunctivitis).

DISCUSSION

The first essential step in ensuring the quality of the final product is the proper identification of the raw drug. All ingredients used in the formulation were authenticated based on the characteristics described in the Ayurvedic Pharmacopoeia of India (API). Phytochemical screening of the sample confirmed the presence of flavonoids, steroids, triterpenoids, and anthraquinones. The physicochemical parameters of the formulation were evaluated and found to be within the acceptable limits specified in the API. The pH analysis indicated that the aqueous solution of the eye drops is nearly neutral in nature.

Using LCMS/MS and GC-MS methods, the phytoconstituents present in *Boerhavia diffusa* were identified and identically matched the authenticated data. The procedure demonstrated linearity for various Quercetin concentrations.

CONCLUSION

The developed Punarnava eye drop formulation is suitable for managing Vataja Abhishyanda (allergic conjunctivitis). Key quality control parameters such as physicochemical properties, TLC fingerprinting, and microbial limit tests were assessed and found to be within acceptable ranges. Based on these findings, it can be concluded that the formulation is patient-compatible and exhibits a stable shelf life under room temperature conditions. This study may serve as a foundation for future research aimed at establishing the safety, therapeutic efficacy, and overall acceptance of the Punarnava eye drop formulation.

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